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(54) Title: SEQUESTRATION OF A $\beta$  IN THE PERIPHERY IN THE ABSENCE OF IMMUNOMODULATING AGENT AS A THERAPEUTIC APPROACH FOR THE TREATMENT OR PREVENTION OF BETA-AMYLOID RELATED DISEASES

(57) Abstract: The present invention describes a method of administering an A $\beta$ -binding agent or drug which has affinity for amyloid beta (A $\beta$ ) in the periphery (blood) and reducing A $\beta$  levels in the brain without the need for the agent or drug to enter the brain itself. The A $\beta$ -binding agents utilized in the methods of the invention are preferably non-immunomodulating agents (e.g., antigenic peptides or antibodies) and bind to A $\beta$  in the periphery, or blood. Such compounds do not significantly cross the blood/brain barrier, and yet they lower amyloid (A $\beta$ ) levels in the brain, thereby serving as safer, therapeutic and prophylactic treatments against diseases associated with A $\beta$  in the brain, e.g., Alzheimer's Disease and amyloid angiopathy, as well as against other AD-related amyloidoses.

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**SEQUESTRATION OF A $\beta$  IN THE PERIPHERY IN THE ABSENCE OF  
IMMUNOMODULATING AGENTS AS A THERAPEUTIC APPROACH FOR  
THE TREATMENT OR PREVENTION OF BETA-AMYLOID RELATED  
DISEASES**

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**FIELD OF THE INVENTION**

The present invention relates to improved drug delivery  
10 methods and the discovery and development of novel compounds and drugs  
for the treatment and prevention of neurological diseases and disorders  
associated with  $\beta$ -amyloid, such as Alzheimer's disease,  $\beta$ -amyloid related  
problems in Down's syndrome and vascular dementia (cerebral amyloid  
angiopathy) and other amyloidosis diseases. The invention further relates to  
15 diagnostic and screening methods for determining or identifying the  
aforementioned diseases and disorders associated with  $\beta$ -amyloid in  
patients.

**BACKGROUND OF THE INVENTION**

Alzheimer's Disease (AD) is the most common cause of  
20 chronic dementia, with approximately two million people in the United States  
having the disease. The histopathologic lesions of Alzheimer's disease (i.e.,  
neuritic amyloid plaques, neurofibrillary degeneration, and granulovascular  
neuronal degeneration) are found in the brains of elderly people with  
Alzheimer's dementia.

25 It is estimated that ten percent of individuals older than 65  
years of age have mild to severe dementia. The number of such lesions  
correlates with the degree of intellectual deterioration. This high prevalence,  
combined with the rate of growth of the elderly segment of the population,

make dementia (and particularly AD) one of the most important of the present-day public health concerns.

An invariant feature of Alzheimer's disease (and AD in Down's syndrome) is the deposition of the small, i.e., approximately 40 to 42 residues, amyloid beta (also referred to as A $\beta$  or Abeta herein) peptide as insoluble  $\beta$ -amyloid plaque in the brain parenchyma. (G.G. Glenner et al., 1984, *Appl. Pathol.*, 2(6):357-69; G.G. Glenner et al., 1984, *Biochem Biophys Res Commun.*, 120(3):885-90; G.G. Glenner et al., 1984, *Biochem Biophys Res Commun.*, 122(3):1131-5). In cerebral amyloid angiopathy, A $\beta$  is deposited in the vasculature. A $\beta$  is generated by proteolysis of the approximately 100 kDa amyloid precursor protein (APP), a broadly expressed type-1 transmembrane protein that is found primarily in the trans-Golgi network (TGN) and at the cell surface (reviewed in B. De Strooper and W. Annaert, 2000, "Proteolytic processing and cell biological functions of the amyloid precursor protein." *J. Cell. Sci.*, 113(Pt 11)(7):1857-1870). The  $\beta$ -amyloid precursor protein APP is further described in D.J. Selkoe et al., 1988, *Proc. Natl. Acad. Sci. USA.*, 85(19):7341-7345; R.E. Tanzi et al., 1988, *Nature*, 331(6156):528-530; and E. Levy et al., 1990, *Science*, 248(4959):1124-1126.

Amyloid plaques containing Abeta (A $\beta$ ) peptides are one of the most significant pathological features of the human Alzheimer's disease brain. Drugs that reduce brain A $\beta$  levels, or remove plaques, are considered to be the most likely to be effective in the treatment or prevention of AD. To date, treatments for AD have focused on the use of anti-A $\beta$  antibodies or peptides which evoke the production of anti-A $\beta$  antibodies, i.e., vaccine therapy. (see, for example, D. Schenk et al., 1999, *Nature*, 400(6740):173-177 and F. Bard et al., 2000, *Nat Med*, 6(8):916-919).

The presumed mode of action for such antibody, or immunomodulatory, treatments is in the clearance of A $\beta$  directly from the brain due to the entry of antibodies into the brain. A side effect of vaccination is an increase of peripheral A $\beta$  levels as levels of A $\beta$  decrease in  
5 the brain, thus resulting in a little understood change in the dynamics between the two systems, brain and periphery.

Vaccination involving anti-A $\beta$  antibodies is a potentially ineffective and possibly even dangerous approach for treatment of AD patients, particularly the elderly who lack (or have less) immune  
10 responsiveness, due to the risk of provoking autoimmune diseases.

Clearance of A $\beta$  from the brain has been reported using immunization of A $\beta$  peptides or passive immunization with anti-A $\beta$  antibodies (D. Schenk et al., 1999, *Nature*, 400(6740):173-177 and F. Bard et al., 2000, *Nature Med.*, 6(8):916-919). The proposed mechanism for this clearance is  
15 microglial phagocytosis carried out by brain immune cells, i.e., microglia, that have been activated by elicited or injected anti-A $\beta$  antibodies.

A goal in the field of therapy and prevention of AD and amyloid-related diseases is the discovery and development of new drugs that are effective due to their mode of action in the periphery, rather than in  
20 the brain, thus obviating the need to enter the brain itself and overcoming the problems encountered in efficient dosage and effectiveness due to the blood/brain barrier. The present invention satisfies this goal by providing new methods of treatment and prevention for AD and other amyloid-related diseases, and by describing new types of drugs and compounds that serve  
25 to treat or prevent disease via the blood.

### SUMMARY OF THE INVENTION

The present invention provides methods and compounds (also termed drugs, substances, reagents, or agents, preferably bioactive agents) employed therein for sequestering A $\beta$  in the blood, or blood components, such as plasma, i.e., the periphery, thereby reducing A $\beta$  levels in the brain for treatment or prevention of beta-amyloid related diseases. The compounds of the invention have an affinity for (i.e., "sequester") A $\beta$ , and bind to and sequester A $\beta$  in the blood, or periphery, e.g., plasma, without needing to enter the brain itself. According to this invention, such compounds do not (and do not need to) cross the blood/brain barrier, and yet they significantly lower amyloid (A $\beta$ ) levels in the brain. Such compounds have been shown in animal models of disease, e.g., a transgenic AD mouse model, to lower A $\beta$  levels in the brain by sequestering A $\beta$  in the periphery, e.g., plasma. That the invention provides a method and drugs used therein which obviate the need for a drug to enter the brain itself, while still significantly lowering amyloid (A $\beta$ ) levels in the brain, offers a great improvement over drugs that currently must enter the brain to have an effect on A $\beta$  levels in the brain.

Thus, it is also an aspect of the present invention to provide a method of treating or preventing AD comprising administering to an individual in need thereof a compound or drug having an affinity for A $\beta$ , which binds to A $\beta$  in the periphery, wherein such a compound or drug, preferably a non-immune related compound or drug, and also preferably, an agent other than an antibody or an immunomodulating agent, sequesters A $\beta$  in the periphery and leads to a reduction in A $\beta$  levels in the brain. For delivery to the periphery, such A $\beta$ -binding compounds are preferably introduced intravenously or subcutaneously; however, any method of

introducing the compound into the blood stream (including via pumps) is acceptable and suitable in accordance with this invention.

It is another aspect of the present invention to provide a method of reducing amyloid (or soluble/insoluble A $\beta$ ) levels in the brain of a patient undergoing treatment by obviating the need to introduce an A $\beta$ -binding drug or compound directly, or indirectly, into the brain. According to the invention, the effectiveness of the method in which the A $\beta$ -binding drug sequesters A $\beta$  in the bloodstream and removes it from the brain is at least as high as a vaccine approach involving the production of antibodies that cross the blood/brain barrier, enter into the brain, and act in the brain.

It is yet a further aspect of the present invention to provide a method for diagnosing, screening, or monitoring treatment of diseases involving  $\beta$ -amyloid or amyloidoses, such as AD, comprising determining an elevation of A $\beta$  levels in the periphery, e.g., plasma, particularly at a time, or series of times, following treatment. In accordance with this aspect of the invention, the elevation of A $\beta$  levels in the periphery, e.g., plasma, serves as a diagnostic marker of diseases involving  $\beta$ -amyloid, particularly, AD.

Further aspects, features and advantages of the present invention will be apparent when considered in connection with the further disclosure of the invention hereinbelow.

#### **DESCRIPTION OF THE INVENTION**

In one of its aspects, the present invention describes compounds (drugs) which have an affinity for, i.e., "sequester", A $\beta$  in the blood, or blood components, e.g., plasma, (periphery) and which reduce A $\beta$  levels in the brain without the need of the compounds (e.g., drugs or bioactive agents) to enter the brain itself. Such compounds sequester A $\beta$  in

the periphery and alter the periphery/brain dynamics so as to reduce A $\beta$  in the brain by virtue of their effective sequestration of A $\beta$  in the periphery.

According to this invention, such compounds are preferably brain impermeable and essentially do not (and do not need to) cross the blood/brain barrier following administration or introduction into a recipient, and yet they significantly lower amyloid (A $\beta$ ) levels in the brain. Also in accordance with this invention, such compounds have been shown in animal models of disease, e.g., AD mouse models, to lower A $\beta$  levels in the brain by sequestering A $\beta$  in the periphery, e.g., plasma. (Example 1). It is thus an aspect of the invention that the A $\beta$  binding agent, drug, compound, and the like, effectively sequesters A $\beta$  in the periphery following administration in the periphery. Preferably, greater than about 50% of the A $\beta$  binding agent, drug or compound remains in the periphery versus the brain following administration in the periphery. More preferably, about 90% or more of the A $\beta$  binding agent, drug or compound remains in the periphery versus the brain following administration in the periphery

In addition, the finding of elevated A $\beta$  in the periphery, particularly in plasma, preferably in conjunction with the administration of agents that bind A $\beta$  and sequester A $\beta$  in the periphery, can serve as a diagnostic marker of  $\beta$ -amyloid-related diseases, especially, AD. The elevation of levels of A $\beta$  in the periphery can further serve as a means of monitoring the effectiveness of treatment of a disease involving A $\beta$ , particularly with a drug or agent that binds and sequesters A $\beta$  in the periphery, thereby leading to its elevation in the periphery. According to this embodiment of the invention, an elevation of A $\beta$  in the periphery reflects an amount of A $\beta$  that is increased relative to that found in normal individuals, such as in plasma, or a base level of A $\beta$ , e.g., in plasma, in individuals who

serve as controls. Determining and/or measuring levels of A $\beta$  can be performed using routine techniques as known in the art, such as radioimmunoassays (RIAs), non-radioactive immunoassays, such as enzyme linked immunoassays (ELISAs), western blotting, dot blotting, mass spectrometry, etc.

Without wishing to be bound by theory, it is proposed that microglial phagocytosis is not necessary for A $\beta$  clearance from the brain in accordance with the present invention. Instead, sequestration in the blood, or blood component, such as plasma, i.e., the periphery, in the absence of an immune modulating agent, by suitable A $\beta$ -binding compounds that are not A $\beta$  peptides or their derivative antibodies, serves to reduce A $\beta$  levels in the brain and to alter the central nervous system (CNS)/periphery dynamics leading to reduction of A $\beta$  in the brain. As used herein, the terms immune modulating agent, and immune related agent, refer to an anti-A $\beta$  antibody or a peptide against some region of Abeta or APP that evokes the production of antibodies, which recognize an Abeta region or APP.

As a consequence of the sequestration of A $\beta$  in the periphery according to this invention, higher levels of A $\beta$  are predicted to be found in the blood (e.g., plasma)/periphery, (see Example 1), because A $\beta$  is sequestered in the blood/periphery by the A $\beta$ -binding compounds. It is also to be understood that the removal of bound A $\beta$ /binding agents by cellular clearance mechanisms may effectively reduce the levels of peripheral A $\beta$  seen following administration of the sequestering agent. The important effect of the methods and reagents of the present invention is that the levels of A $\beta$  found in the brain as a result of keeping A $\beta$  sequestered in the periphery are reduced, which is advantageous for the therapeutic effect of the method and compounds of the present invention.

The invention also allows for determining or monitoring a drug's effectiveness by monitoring A $\beta$  levels in the periphery, such as in the plasma, instead of, or in addition to, brain A $\beta$  levels. (see, e.g., Example 1). Methods of monitoring A $\beta$  levels in an individual undergoing drug treatment  
5 or therapy for amyloid related diseases involve determining the levels of A $\beta$  in the individual's peripheral body fluid sample, e.g., plasma, at one or more time intervals following treatment or therapy involving an A $\beta$  binding agent that sequesters A $\beta$  in the periphery. For example, an individual can be monitored at about 1-25 hours, preferably at about 2-10 hours following  
10 administration of the A $\beta$  binding agent, or at varying time intervals therebetween, to determine if A $\beta$  levels are elevated. Such monitoring methods are particularly useful for determining if a given drug treatment is beneficial, or to determine if doses of a drug or a drug combination should be modified or adjusted during the course of treatment. In such methods, it  
15 is preferred to use the individual's pretreatment levels of A $\beta$  in the periphery to compare and assess treatment and post-treatment levels of A $\beta$  in the periphery. Controls can also include peripheral levels of A $\beta$  in disease-free (or dementia-free) individuals, as well as peripheral levels of A $\beta$  in individuals having an amyloid related disease, e.g., AD. Human plasma and  
20 cerebrospinal fluid levels of amyloid beta proteins, particularly, A $\beta$ 40 and A $\beta$ 42, have been reported (see, e.g., Mehta et al., 2000, *Arch. Neurol.*, 57:100-105).

According to this invention, A $\beta$  levels can be measured in an individual's body fluid sample, such as blood, serum, or plasma, using  
25 conventionally known assays that detect A $\beta$ , for example, radioisotopic immunoassays or non-isotopic immunoassays, e.g., fluorescent immunoassays, chemiluminescent immunoassays and enzymatic immunoassays, such as an enzyme linked immunoassay (ELISA), as are

- commercially available, known and practiced in the art, for example, Beta-amyloid (A $\beta$ ) [1-40] Immunoassay (Biosource, Camarillo, CA; Cat. No. KHB3481); Beta-amyloid (A $\beta$ ) [1-42] Immunoassay (Biosource, Camarillo, CA; Cat. No. KHB3441); and Human Amyloid beta (1-40)
- 5 Immunoassay (IBL, Fujioka, Gunma, Japan; Cat. No. 17713).

Typically, an ELISA assay initially involves preparing, obtaining, or employing an antibody specific to A $\beta$ , preferably a monoclonal antibody. In addition, a reporter antibody is used. In some ELISA protocols, the reporter antibody recognizes and binds to the anti-A $\beta$ -specific

10 monoclonal antibody. To the reporter antibody is attached a detectable reagent such as a radioactive isotope, a fluorescent moiety, a chemiluminescent moiety, or, in an ELISA, an enzyme, such as horseradish peroxidase or alkaline phosphatase.

As is appreciated by those skilled in the art, ELISAs can be

15 performed in a number of assay formats. In one ELISA format, a host sample, e.g., a patient body fluid sample, is incubated on a solid support, e.g., the wells of a microtiter plate, or a polystyrene dish, to which the proteins in the sample can bind. Any free protein binding sites on the dish are then blocked by incubating with a non-specific protein such as bovine

20 serum albumin. The monoclonal antibody is then added to the solid support, e.g., the wells or the dish, and allowed to incubate. During the incubation time, the monoclonal antibodies attach to any A $\beta$  polypeptides or peptides that have attached to the polystyrene dish.

All unbound monoclonal antibody is washed away using an

25 appropriate buffer solution. The reporter antibody, e.g., linked to horseradish peroxidase, is added to the support, thereby resulting in the binding of the reporter antibody to any monoclonal antibody which has bound to A $\beta$  present in the sample. Unattached reporter antibody is then

washed away. Peroxidase substrate is added to the support and the amount of color developed in a given time period provides a measurement of the amount of A $\beta$  that is present in a given volume of individual or patient sample when compared to a standard curve.

- 5                    In another ELISA format, antibody specific for a particular analyte is attached to the solid support, i.e., the wells of a microtiter plate or a polystyrene dish, and a sample containing analyte is added to the substrate. Detectable reporter antibodies, which bind to the analyte that has bound to the capture antibodies on the support, are then added, after the  
10 appropriate incubations and washings, and analyte-antibody complexes are detected and quantified.

- The present invention also embraces a sandwich type ELISA immunoassay typically performed using microtiter plates. A capture antibody, that can be polyclonal or monoclonal, preferably a monoclonal  
15 antibody, that specifically recognizes an epitope in the A $\beta$  peptide is used, along with a labeled detector antibody, e.g., an alkaline phosphatase-labeled antibody, or a horse radish peroxidase-labeled antibody, preferably a monoclonal antibody. The detector antibody also specifically recognizes an epitope in A $\beta$ . Preferably also, the capture antibody does not inhibit  
20 binding to A $\beta$ . The production of both polyclonal and monoclonal antibodies, particularly monoclonal antibodies that are specific for A $\beta$ , is performed using techniques and protocols that are conventionally known and practiced in the art.

- In a particular embodiment according to this invention, a  
25 capture anti-A $\beta$  antibody of the assay method is immobilized on the interior surface of the wells of the microtiter plate. To perform the assay, an appropriate volume of sample is incubated in the wells to allow binding of the antigen by the capture antibody. The immobilized antigen is then

exposed to the labeled detector antibody. Addition of substrate to the wells, if the detectable label is alkaline phosphatase, for example, allows the catalysis of a chromogen, i.e., *para*-nitrophenylphosphate (pNPP), if the label is alkaline phosphatase, into a colored product. The intensity of the colored product is proportional to the amount of A $\beta$  that is bound to the microtiter plate.

Standards are used to allow accurate quantitative determinations of A $\beta$  in the samples undergoing analysis. A microtiter plate reader simultaneously measures the absorbance of the colored product in the standard and the sample wells. Correlating the absorbance values of samples with the standards run in parallel in the assay allows the determination of the levels of A $\beta$  in the sample. Samples are assigned a quantitative value of A $\beta$  in nanograms per milliliter (ng/ml) of blood, serum, plasma, or other body fluid.

The present invention provides a significant advantage to the treatment and prevention of AD and amyloid-related diseases in that drugs and active compounds according to this invention are not required to cross the blood/brain barrier to exert their effect. Having to cross the blood/brain barrier is an enormous obstacle to developing effective drugs for use in the brain. This invention overcomes this obstacle. One of the major differences between this method and others is that it uses a non-antibody compound, or a compound that is not related to an antibody, to achieve the sequestration of A $\beta$ , and that this sequestration has its primary effect in the periphery. A consequence of the method and the compounds utilized therein is a decrease in A $\beta$  in the brain.

In addition, a second advantage of this invention is that neither A $\beta$  peptides, nor anti-A $\beta$  antibodies, is administered to a host, thus negating the risk of an adverse immune response, or the lack of an effective immune

response. For AD and amyloid angiopathy, this method preferably involves the use of A $\beta$ -binding compounds and drugs of the invention, more preferably formulated as pharmaceutically acceptable compositions as described herein.

5                   The method further comprises the administration of an amyloid beta (A $\beta$ )-binding compound, agent, or drug in the periphery of an individual in need thereof, wherein the compound sequesters A $\beta$  in the periphery and concomitantly decreases A $\beta$  levels in the brain of the individual undergoing treatment. According to the method of the present invention, the need to  
10   Introduce an agent or drug directly into the brain, or to have the drug cross the blood/brain barrier is obviated. Also according to this method, brain A $\beta$  levels are reduced, due to the effects of the A $\beta$ -binding drugs and compounds described herein on A $\beta$  in the periphery.

                  The method and A $\beta$ -binding compounds and agents used  
15   therein in accordance with the present invention are suitable for the treatment, both prophylactic and therapeutic, of neurological diseases and disorders associated with  $\beta$ -amyloid, such as Alzheimer's disease,  $\beta$ -amyloid related problems in Down's syndrome and vascular dementia (cerebral amyloid angiopathy) (A.J. Rozemuller et al., 1993, *Am. J. Pathol.*,  
20   142(5):1449-1457) and other amyloidosis diseases. The method involving peripheral sequestration of disease associated agents, e.g., peptides, or proteins or aggregates thereof, by non-immunomodulating agents that bind to such disease associated agents in the blood/periphery in accordance with the present invention, are also useful in the treatment or prevention of other  
25   cortical or vascular amyloidoses, including those caused by cystatin C (ACys), prion protein (AScr), transthyretin (ATTR), gelsolin (AGel), and Amyloid ABri (or A-WD) (see, M. Yamada, 2000, "Cerebral amyloid

angiopathy: an overview", *Neuropathology*, 20(1):8-22). Cortical or vascular amyloidoses are very similar in etiology to AD.

In accordance with the present invention, the method of delivering A $\beta$ -binding drugs to the periphery has been shown to be at least  
5 as effective as the vaccine approach in transgenic mouse models, if not more so. Indeed, the use of non-toxic, non-immune related compounds and drugs can overcome adverse immune responses that are frequently associated with the use of brain-directed immunovaccines. Prior to the present invention, the treatment of brain amyloidosis by administering non-  
10 immune related A $\beta$ -binding agents in the periphery and sequestering or "locking away" A $\beta$  in the blood, or periphery, has not been shown.

Suitable compounds that can be employed in the method of this invention include, but are not limited to, small molecules, e.g., peptides, proteins; biologic agents; and drugs that have an affinity for A $\beta$  and bind A $\beta$   
15 in the periphery. Such compounds, molecules, agents and drugs have an A $\beta$ -binding domain that physically binds to and locks away A $\beta$  in the periphery. The compound, molecule, agent or drug can bind to or have affinity for a variety of A $\beta$  peptides, e.g., A $\beta$  peptides derived from APP; A $\beta$  peptides of different fragment lengths, e.g., A $\beta$ 40 or A $\beta$ 42, and the like. In  
20 addition, the compound, molecule, agent or drug can bind to or have affinity for any portion of an A $\beta$  peptide, e.g., the N- or C- terminus, or other regions of the molecule. Non-immune related and/or non-immunomodulatory compounds or drugs are preferred. Most preferably the compounds are non-toxic and well tolerated following their use in the treatment and  
25 prevention methods.

An advantage of the use of molecules other than immune related compounds, such as antibodies, for peripheral sequestration of

Abeta (A $\beta$ ) is that non-antibody related drugs can be manipulated more easily than antibodies. For example, sequestering compounds can be modified to be metabolized faster by the addition of certain chemical structures, as known and practiced in the art. For example, sequestering  
5 compounds can be modified by the addition of side chain(s) which can modulate metabolism. Such chemical modification of the non-antibody A $\beta$ -binding and sequestering compounds can improve their efficacy and reduce toxicity and/or potentially adverse side effects. The derivative of Congo Red, an A $\beta$ -Imaging agent, as described herein, is particularly suitable for  
10 chemical derivatization or modification.

Nonlimiting examples of such A $\beta$ -binding compounds include compounds having an affinity for A $\beta$ , particularly, cortigangliosides, such as GM1, the actin-regulating molecule gelsolin, particularly, the extracellular A $\beta$ -binding domain of gelsolin, and A $\beta$  staining molecules, such as  
15 derivatives of Congo Red, e.g., [1,4-bis(3-carboxy-4-hydroxyphenylethenyl)-benzene and 5,5'-[(1,1' biphenyl)-4, 4'-diylbis(azo)] bis [2-hydroxybenzoic acid] disodium salt (chrysamine-G or CG), as described in U.S. Patent No. 6,133,259 and WO 96/34853. A preferred A $\beta$  staining molecule is the A $\beta$  staining dye compound Chrysamine-G, as described in U.S. Patent No.  
20 6,133,259 and WO 96/34853. (Example 2). Other nonlimiting examples of A $\beta$  binding agents that are suitable for use in the methods of this invention include A $\beta$  imaging agents (e.g., Klunk et al., 1995, "Chrysamine-G binding to Alzheimer and control brain: autopsy study of a new amyloid probe", *Neurobiol. Aging*, 16: 541-548),  $\beta$ -sheet breakers (e.g., Bohrmann et al.,  
25 2000, "Self-assembly of beta-amyloid 42 is retarded by small molecular ligands at the stage of structural intermediates", *J. Struct. Biol.*, 130:232-246),  $\beta$ -sheet formation inhibitors (e.g., Findels et al., 1999, "Modified-peptide inhibitors of amyloid beta-peptide polymerization", *Biochemistry*,

38:6791-6800), and the like, are encompassed for use in the present invention.

The A $\beta$ -binding compounds according to the invention can be incorporated into pharmaceutical formulations, or pharmaceutical  
5 compositions, preferably physiologically acceptable compositions, according to known methods, such as by admixture with a pharmaceutically acceptable carrier, diluent, or excipient. One or more A $\beta$ -binding compounds or drugs comprise the pharmaceutical compositions and are formulated as active ingredients in the compositions in a therapeutic or  
10 prophylactic amount.

The pharmaceutically, or physiologically, acceptable carrier, diluent, or excipient can be any compatible non-toxic substance suitable to deliver the compound to a host or recipient. Sterile water, alcohol, fats, waxes and inert solids may be used as carriers. In addition,  
15 pharmaceutically acceptable adjuvants, buffering agents, dispersing agents, and the like, may also be incorporated into the pharmaceutical compositions. The preparation of pharmaceutical compositions comprising active agents is well described in the scientific and medical literature. Examples of methods of formulation, and carriers, etc. may be found in the  
20 latest edition of *Remington's Pharmaceutical Sciences*, 18th Ed., 1990, Mack Publishing Co, Easton, PA.

To formulate a pharmaceutically acceptable composition suitable for effective administration, preferably *in vivo*, or even *ex vivo*, such compositions will contain an effective amount of the active compound,  
25 biomolecule, agent or drug. Pharmaceutical compositions of the present invention are administered to an individual in amounts effective to treat or prevent AD, amyloid angiopathy, or other A $\beta$ -associated diseases or conditions. The effective amount may vary according to a variety of factors,

such as an individual's physical condition, weight, sex and age. Other factors include the mode and route of administration. These factors are realized and understood by the skilled practitioner and are routinely taken into account when administering a therapeutic agent to an individual.

5                   Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective and sufficient amount to directly bind A $\beta$  in the periphery, sequester it there, and reduce the A $\beta$  levels in the brain. The determination of an effective dose is well within the capability of the skilled practitioner in  
10 the art. The therapeutically effective dose can be estimated initially either in cell culture assays, or in animal models, usually mice, rabbits, dogs, pigs, rats, monkeys, or guinea pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in  
15 humans.

A therapeutically effective dose refers to that amount of the A $\beta$ -binding compound or drug which ameliorates, reduces, or eliminates the symptoms or condition. In accordance with this invention, the effective dose is preferably that which lowers, reduces, or eliminates levels of A $\beta$ , or  
20 buildup of A $\beta$ , in the brain, while binding to and "locking up" A $\beta$  in the periphery. The exact dosage is chosen in view of the patient to be treated, the route of administration, the severity of disease, and the like.

The concentration of the A $\beta$ -binding drug, compound or bioactive agent in the pharmaceutical carrier may vary, e.g., from less than  
25 about 0.1% by weight of the pharmaceutical composition to about 20% by weight, or greater. As a nonlimiting example, a typical pharmaceutical composition for intramuscular administration would be formulated to contain one to four milliliters (ml) of sterile buffered water and one microgram ( $\mu$ g) to

one milligram (mg) of the A $\beta$ -binding drug or compound of the present invention. A typical composition for intravenous infusion could be formulated to contain, for example, 100 to 500 ml of sterile buffered water or Ringer's solution and about 1 to 100 mg of the A $\beta$ -binding drug or  
5 compound.

The daily dosage of the pharmaceutical, or physiologically acceptable, products may be varied over a wide range, for example, from about 0.01 to 1,000 mg per adult human/per day. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0001 mg/kg to  
10 about 100 mg/kg of body weight per day. The range is more particularly from about 0.001 mg/kg to 10 mg/kg of body weight per day. Even more particularly, the range varies from about 0.05 to about 1 mg/kg. Of course, it will be understood by the skilled practitioner that the dosage level will vary depending upon the potency or effectiveness of a particular compound, or  
15 combination of compounds, and that certain compounds will be more potent or effective than others.

In addition, the dosage level will vary depending upon the bioavailability of the compound. The more bioavailable and potent the compound, the less amount of the compound will need to be administered  
20 through any delivery route, including, but not limited to, oral delivery. The dosages of the A $\beta$ -binding compounds are adjusted, if combined, in order to achieve desired effects. On the other hand, dosages of the various A $\beta$ -binding agents or compounds may be independently optimized and combined to achieve a synergistic result, wherein the pathology is reduced  
25 more than it would be if one single agent or compound were used alone.

The pharmaceutical compositions may be provided to an individual in need of therapeutic treatment by a variety of routes, such as; for example, subcutaneous, topical, oral, intraperitoneal, intradermal,

intravenous, intranasal, rectal, intramuscular, and within the pleural cavity. Administration of pharmaceutical compositions is accomplished orally or parenterally. More specifically, methods of parenteral delivery include topical, intra-arterial, intramuscular, subcutaneous, intramedullary,  
5 intrathecal, intraventricular, intravenous, intraperitoneal, intranasal administration, or via the pleural cavity. In addition, the compounds according to the invention can be delivered via one or more routes of administration through the use of pumps.

Also embraced are transdermal modes of delivery, such as  
10 patches and the like, with or without a suitable permeation enhancer. The methods and compositions embodied by the invention provide a means by which one or more of the A $\beta$ -binding drugs, or medicaments, can be effectively administered in a transdermal system. Frequently, compounds having poor topical absorption, or which are required at high dosage levels,  
15 are delivered transdermally. Accordingly, a transdermal means of delivering a drug composition (often with a permeation enhancing composition) to the skin is that of the transdermal patch or a similar device as known and described in the art. Examples of such devices are disclosed in U.S. Patent Nos. 5,146,846, 5,223,262, 4,820,724, 4,379,454 and 4,956,171. The  
20 transdermal mode of storing and delivering the compositions onto the skin and forming the active composition is convenient and well suited for the purposes of the invention.

The present invention also provides suitable topical, oral,  
systemic and parenteral pharmaceutical formulations for use in the methods  
25 of treatment described herein. It is to be appreciated that the compositions containing the A $\beta$ -binding compounds can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as

tablets or capsules (including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, the therapeutic compounds may also be administered in intravenous (both bolus  
5 and infusion), intraperitoneal, subcutaneous, topical (with or without occlusion), or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. The preferred mode of delivery for the A $\beta$ -binding compounds according to the present invention is intravenous.

10 For topical administration, the compositions of the present invention may be formulated in oil, water, or combinations thereof. Preferred is a dermatologically acceptable formulation comprising an oil-in-water emulsion. Examples of other dermatologically acceptable vehicle formulations of the present invention include, but are not limited to, any  
15 suitable non-toxic or pharmaceutically acceptable topical carrier, such as a solution, suspension, emulsion, lotion, ointment, cream, gel, plaster, patch, film, tape or dressing preparation, all of which are well-known to those skilled in the art of topical skin formulations and preparations.

The pharmaceutical compositions of the present invention can  
20 be administered for therapeutic and/or for prophylactic purposes of treating diseases, pathologies, or conditions related to the increase in A $\beta$  levels, or the deposition of A $\beta$  in the brain, for example, AD and amyloid angiopathy. Prophylactic treatment is preferred, although therapeutic treatment is also efficacious. For prophylactic applications, the pharmaceutical compositions  
25 of this invention are administered to an individual who is susceptible to, or prone to, the disease, pathology, or condition. Such individuals can be identified by genetic screening and/or clinical analysis, such as is described in the medical literature (see, e.g., Goate, 1991, *Nature*, 349:704-706 and

E.H. Corder et al., 1993, *Science*, 261(5123):921-923). In such cases, the pharmaceutical compositions bind to or sequester A $\beta$  in the periphery at a symptomatically early stage, thus preferably preventing either the initial stages of, or the severity of, disease progression. Furthermore, prophylactic  
5 treatment can be applied to any individual wishing to undertake treatment, regardless of their susceptibility.

In therapeutic applications, the pharmaceutical compositions of this invention are administered to an individual in need thereof; such individuals already suffer from, or are thought to suffer from the disease,  
10 pathology, or condition. In general, a dose of an A $\beta$ -binding compound effective for prophylactic treatment or therapy is the same as that for therapeutic treatment or therapy.

### **EXAMPLES**

The following example describes specific aspects of the  
15 invention to illustrate the invention and provides a description of the present methods for those of skill in the art. The example should not be construed as limiting the invention, as the examples merely provide specific methodology useful in the understanding and practice of the invention and its various aspects.

#### **Example 1**

Transgenic mice that develop AD-related amyloidosis, (i.e., PS/APP mice; See, L. Holcomb et al., 1998, *Nature Med.*, 4(1):97-100) were used for the studies described in this Example to assess how peripheral sequestration of A $\beta$  affected brain A $\beta$  levels. The ganglioside GM1 was  
25 utilized as an exemplary A $\beta$ -binding compound, since GM1 is known to bind A $\beta$  strongly, and does not appear to enter the brain. In addition, a second compound, gelsolin, which is too large to cross the blood/brain barrier (BBB), and is completely unrelated to GM1, but which is also known to bind

A $\beta$  with great avidity, was administered peripherally to confirm the universality of the mechanism.

PS/APP mice were injected every two days for two weeks either with GM1 (number of mice =6) (15 mg/kg, ip.), with gelsolin (number of mice =3) (60  $\mu$ g/kg, ip), or with vehicle, (phosphate buffered saline), (number =7), into the periphery at 9 weeks of age, an age when amyloid pathology in the brain is not visible. The mice were left for 1 week without injections (the "wash-out" period) and were then sacrificed at 12 weeks of age, an age when amyloid deposition has been initiated and measurable levels of A $\beta$  are present in the vehicle treated controls.

The levels of A $\beta$  in the peripheral blood were tested at three time points during the drug administration period (i.e., after 1 week of injections; after two weeks of injections; and after the wash-out period). Data for the third time point only are shown. The levels of A $\beta$  peptides (A $\beta$ 40 or A $\beta$ 42) in the brain and plasma were assessed by ELISA assay. All of the A $\beta$  in the brain (including A $\beta$  in plaques) was extracted in 70% formic acid (FA). The levels of A $\beta$  peptides in GM1 or gelsolin treated mice were compared with those of vehicle treated controls.

The results presented in Table 1 show that there was a statistically significant ( $p < 0.05$ ) decrease in both A $\beta$ 40 and A $\beta$ 42 in the FA-soluble brain fraction in GM1-treated mice compared with those in control animals. This correlates with a statistically significant increase in peripheral A $\beta$ 40 and A $\beta$ 42 at the same time point. For gelsolin, there was also a significant decrease in A $\beta$ 42, which correlated with a statistically significant increase in peripheral A $\beta$ , thus confirming that the general principle of A $\beta$  sequestration in the periphery being linked to reduction of A $\beta$  in the CNS

holds true for very different types of compounds that have the unifying property of being able to bind A $\beta$  in the blood.

**Table 1**

5 Changes of brain Abeta load [fmole/mg protein] in GM1- and Gelsolin-treated mice compared with controls (vehicle)

Treatment	n	Abeta40	Abeta42
Vehicle	7	3630 $\pm$ 30	4100 $\pm$ 200
GM1	6	2147 $\pm$ 226	2990 $\pm$ 410
	%	59	73
P-value	p	0.005**	0.031*
Gelsolin	3	3473 $\pm$ 673	2473 $\pm$ 167
	%	96	60
P-value	p	0.502	0.021*

10 \*: results statistically significant to the 5% level (p=0.05);

10 \*\*: results statistically significant to the 1% level (p=0.001).

10

The results presented in Table 2 show the changes in plasma load of both A $\beta$ 40 and A $\beta$ 42 in GM1-treated mice compared with control (vehicle) animals. As can be observed, there was a significant increase in plasma Abeta levels. For GM1, both Abeta40 and Abeta42 are increased; for gelsolin, Abeta42 is increased to a greater extent than is Abeta40. Thus, the effect with gelsolin may reflect a different preference for Abeta42 over Abeta40.

**Table 2**

20 Changes of plasma Abeta load [fmole/ml plasma] in GM1 treated mice compared with controls (vehicle)

Treatment	n	Abeta40	Abeta42
Vehicle	7	100 $\pm$ 21	100 $\pm$ 7
GM1	6	177 $\pm$ 29	124 $\pm$ 11
	p	0.021**	0.036*
Gelsolin	3	218 $\pm$ 87	153 $\pm$ 35
	p	0.016**	0.039*

20 \*: results statistically significant to the 5% level (p=0.05);

20 \*\*: results statistically significant to the 1% level (p=0.001).

Neither GM1 nor gelsolin is known to cross into the brain from the periphery to any degree. In addition, as part of the studies related to those described in this example, GM1 was introduced directly into the brain of transgenic mice, but no change in A $\beta$  levels was observed. Thus, the

5 results indicate that the effects of GM1 administration in the test mice is due to the sequestration of A $\beta$  in the periphery, thereby leading to a change in dynamics between brain and peripheral A $\beta$  transport. This is the first time that such a result has been shown for a peripherally administered compound that is not an antibody. As such, the invention affords a significant

10 advantage to the art by describing and promoting A $\beta$ -binding compounds that require neither penetration of the brain nor the evocation of an immune response, which are potentially harmful and ineffective ways to modulate the risk of AD in human patients.

It will be appreciated that the use of GM1 or gelsolin in the

15 present example is not limiting to the types of compounds considered to be suitable for use in the present invention. Indeed, in accordance with this invention, any A $\beta$  binding molecule can have the same effect following peripheral administration, thus providing a powerful treatment and therapeutic for AD sufferers, as well as those afflicted with other

20 amyloidoses, e.g., amyloid angiopathy.

### **Example 2**

In this Example, as in Example 1, transgenic mice that develop AD-related amyloidosis, (i.e., PS/APP mice; See, L. Holcomb et al., 1998,

25 *Nature Med.*, 4(1):97-100) were used to assess the A $\beta$ -binding compound chrysamine G (CG) in the peripheral sequestration of A $\beta$  according to this invention, and to determine how the peripheral sequestration of A $\beta$  by this compound affected brain A $\beta$  levels. CG is known to bind A $\beta$  strongly, and is less brain permeable than GM1.

PS/APP mice at 10 weeks of age were injected once either with CG (number of mice=3, dosage: 20 mg/kg) or vehicle (phosphate buffered saline, number of mice=2) into the blood stream. Blood samples were collected prior to treatment (injection) and post-treatment at 10 minutes, 2.5 hours, 5 hours and 25 hours after injection. Blood A $\beta$  levels were compared between pre-treatment versus post-treatment at 10 minutes, 2.5, 5 and 25 hours after injection. The levels of A $\beta$  peptides (A $\beta$ 40 or A $\beta$ 42) in the plasma were assessed by ELISA immunoassay. The levels of A $\beta$  peptides in the periphery, i.e., plasma, of CG treated mice were compared with plasma A $\beta$  levels in pre-treatment mice at various time points.

Changes in plasma A $\beta$  levels after injection with CG were compared with pre-treatment plasma A $\beta$  levels as shown in Table 3.

**Table 3**

15

Changes in plasma A $\beta$ levels after injection with CG	
Hours after injection	A $\beta$ 42 level [% of pre-treatment time point]
2.5	110 $\pm$ 8 ( $p=0.232$ )
5	125 $\pm$ 8 ( $p=0.049$ )
7.5	125 $\pm$ 7 ( $p=0.034$ )
24	112 $\pm$ 13 ( $p=0.341$ )
48	111 $\pm$ 10 ( $p=0.256$ )

The results presented in Table 3 show that there was a statistically significant ( $p<0.05$ ) increase in A $\beta$ , as represented by A $\beta$ 42 determination, in the plasma of the mice injected with CG at 5 and 7.5 hours after injection. Changes in plasma load of A $\beta$ 42 after injection with CG were compared to the plasma A $\beta$  level at pre-treatment time points. As can be observed, there was a significant increase in plasma A $\beta$  levels after injection of CG.

Following a one-week wash out period, the effect in brain A $\beta$  level after continuous injection was examined. PS/APP mice were injected every day for one week into the periphery, with either CG (number of mice = 3) (20 mg/kg, ip.) or vehicle (phosphate buffered saline) (number of mice = 2), at 11 weeks of age, an age when amyloid pathology in the brain is not visible. The mice were sacrificed at 12 weeks of age, an age when amyloid deposition has been initiated and measurable levels of A $\beta$  are present in the vehicle treated controls.

The levels of A $\beta$  in the peripheral blood were tested at the end of the administration period. The levels of A $\beta$  peptides (e.g., A $\beta$ 40 or A $\beta$ 42) in the brain and plasma were assessed by ELISA assay. All of the A $\beta$  in the brain (including A $\beta$  in plaques) was extracted in 70% formic acid (FA). The levels of A $\beta$  peptides in CG treated mice were compared with those of vehicle treated controls. The results presented in Tables 4 and 5 show that there was a statistically significant ( $p < 0.05$ ) decrease in A $\beta$ 40 and/or A $\beta$ 42 in the FA-soluble brain fraction in CG-treated mice compared with those in control animals tested 1 week after injection. This correlates with a statistically significant increase in peripheral A $\beta$ 40 and A $\beta$ 42 at the same time point.

20

**Table 4**Change of plasma A $\beta$  levels 1 week following injection with CG

	Plasma A $\beta$ 42 level [% of control]
Vehicle	100 $\pm$ 9
CG treated	331 $\pm$ 10
	( $p=0.0081$ )

**Table 5**Change of brain A $\beta$  1week following Injection with CG

	A $\beta$ 40 [fmol/ml]	A $\beta$ 42 [fmol/ml]
Vehicle	926 $\pm$ 26	1209 $\pm$ 292
CG	541 $\pm$ 50	540 $\pm$ 271
	(P=0.0031)	(P=0.2038)*

\*: Because of data variation, A $\beta$ 42 was not statistically significant, p=0.20.

5                   The method of the present invention for determining elevated levels of A $\beta$  in the periphery for the purposes of diagnosing, screening, or monitoring patient treatment, treatment outcome, or the course and/or severity of amyloid-related disease in an individual preferably involves a pretreatment or baseline value for assessing peripheral elevation of A $\beta$

10                   levels in the individual undergoing testing. In the examples presented herein, an elevation of plasma Abeta was compared by percentage pre-treatment time point of an individual animal. Similar comparative assessments of pretreatment and treatment Abeta levels can be employed for the testing of other mammals, including humans, particularly because the

15                   range of Abeta levels can be large between and among individuals. As a nonlimiting guide, a representative non-elevated level of Abeta in human plasma (e.g., periphery) is about 25%, as determined experimentally (e.g., Mehta et al., 2000, *ibid.*).

20                   The contents of all patents, patent applications, published PCT applications and articles, books, references, reference manuals and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the invention pertains.

25                   As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present invention, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive

such as an individual's physical condition, weight, sex and age. Other factors include the mode and route of administration. These factors are realized and understood by the skilled practitioner and are routinely taken into account when administering a therapeutic agent to an individual.

5                   Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective and sufficient amount to directly bind A $\beta$  in the periphery, sequester it there, and reduce the A $\beta$  levels in the brain. The determination of an effective dose is well within the capability of the skilled practitioner in  
10 the art. The therapeutically effective dose can be estimated initially either in cell culture assays, or in animal models, usually mice, rabbits, dogs, pigs, rats, monkeys, or guinea pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in  
15 humans.

                  A therapeutically effective dose refers to that amount of the A $\beta$ -binding compound or drug which ameliorates, reduces, or eliminates the symptoms or condition. In accordance with this invention, the effective dose is preferably that which lowers, reduces, or eliminates levels of A $\beta$ , or  
20 buildup of A $\beta$ , in the brain, while binding to and "locking up" A $\beta$  in the periphery. The exact dosage is chosen in view of the patient to be treated, the route of administration, the severity of disease, and the like.

                  The concentration of the A $\beta$ -binding drug, compound or bioactive agent in the pharmaceutical carrier may vary, e.g., from less than  
25 about 0.1% by weight of the pharmaceutical composition to about 20% by weight, or greater. As a nonlimiting example, a typical pharmaceutical composition for intramuscular administration would be formulated to contain one to four milliliters (ml) of sterile buffered water and one microgram ( $\mu$ g) to

one milligram (mg) of the A $\beta$ -binding drug or compound of the present invention. A typical composition for intravenous infusion could be formulated to contain, for example, 100 to 500 ml of sterile buffered water or Ringer's solution and about 1 to 100 mg of the A $\beta$ -binding drug or  
5 compound.

The daily dosage of the pharmaceutical, or physiologically acceptable, products may be varied over a wide range, for example, from about 0.01 to 1,000 mg per adult human/per day. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0001 mg/kg to  
10 about 100 mg/kg of body weight per day. The range is more particularly from about 0.001 mg/kg to 10 mg/kg of body weight per day. Even more particularly, the range varies from about 0.05 to about 1 mg/kg. Of course, it will be understood by the skilled practitioner that the dosage level will vary depending upon the potency or effectiveness of a particular compound, or  
15 combination of compounds, and that certain compounds will be more potent or effective than others.

In addition, the dosage level will vary depending upon the bioavailability of the compound. The more bioavailable and potent the compound, the less amount of the compound will need to be administered  
20 through any delivery route, including, but not limited to, oral delivery. The dosages of the A $\beta$ -binding compounds are adjusted, if combined, in order to achieve desired effects. On the other hand, dosages of the various A $\beta$ -binding agents or compounds may be independently optimized and combined to achieve a synergistic result, wherein the pathology is reduced  
25 more than it would be if one single agent or compound were used alone.

The pharmaceutical compositions may be provided to an individual in need of therapeutic treatment by a variety of routes, such as; for example, subcutaneous, topical, oral, intraperitoneal, intradermal,

intravenous, intranasal, rectal, intramuscular, and within the pleural cavity. Administration of pharmaceutical compositions is accomplished orally or parenterally. More specifically, methods of parenteral delivery include topical, intra-arterial, intramuscular, subcutaneous, intramedullary, 5 intrathecal, intraventricular, intravenous, intraperitoneal, intranasal administration, or via the pleural cavity. In addition, the compounds according to the invention can be delivered via one or more routes of administration through the use of pumps.

Also embraced are transdermal modes of delivery, such as 10 patches and the like, with or without a suitable permeation enhancer. The methods and compositions embodied by the invention provide a means by which one or more of the A $\beta$ -binding drugs, or medicaments, can be effectively administered in a transdermal system. Frequently, compounds having poor topical absorption, or which are required at high dosage levels, 15 are delivered transdermally. Accordingly, a transdermal means of delivering a drug composition (often with a permeation enhancing composition) to the skin is that of the transdermal patch or a similar device as known and described in the art. Examples of such devices are disclosed in U.S. Patent Nos. 5,146,846, 5,223,262, 4,820,724, 4,379,454 and 4,956,171. The 20 transdermal mode of storing and delivering the compositions onto the skin and forming the active composition is convenient and well suited for the purposes of the invention.

The present invention also provides suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the methods 25 of treatment described herein. It is to be appreciated that the compositions containing the A $\beta$ -binding compounds can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as

tablets or capsules (including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, the therapeutic compounds may also be administered in intravenous (both bolus  
5 and infusion), intraperitoneal, subcutaneous, topical (with or without occlusion), or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. The preferred mode of delivery for the A $\beta$ -binding compounds according to the present invention is intravenous.

10 For topical administration, the compositions of the present invention may be formulated in oil, water, or combinations thereof. Preferred is a dermatologically acceptable formulation comprising an oil-in-water emulsion. Examples of other dermatologically acceptable vehicle formulations of the present invention include, but are not limited to, any  
15 suitable non-toxic or pharmaceutically acceptable topical carrier, such as a solution, suspension, emulsion, lotion, ointment, cream, gel, plaster, patch, film, tape or dressing preparation, all of which are well-known to those skilled in the art of topical skin formulations and preparations.

The pharmaceutical compositions of the present invention can  
20 be administered for therapeutic and/or for prophylactic purposes of treating diseases, pathologies, or conditions related to the increase in A $\beta$  levels, or the deposition of A $\beta$  in the brain, for example, AD and amyloid angiopathy. Prophylactic treatment is preferred, although therapeutic treatment is also efficacious. For prophylactic applications, the pharmaceutical compositions  
25 of this invention are administered to an individual who is susceptible to, or prone to, the disease, pathology, or condition. Such individuals can be identified by genetic screening and/or clinical analysis, such as is described in the medical literature (see, e.g., Goate, 1991, *Nature*, 349:704-706 and

E.H. Corder et al., 1993, *Science*, 261(5123):921-923). In such cases, the pharmaceutical compositions bind to or sequester A $\beta$  in the periphery at a symptomatically early stage, thus preferably preventing either the initial stages of, or the severity of, disease progression. Furthermore, prophylactic  
5 treatment can be applied to any individual wishing to undertake treatment, regardless of their susceptibility.

In therapeutic applications, the pharmaceutical compositions of this invention are administered to an individual in need thereof; such individuals already suffer from, or are thought to suffer from the disease,  
10 pathology, or condition. In general, a dose of an A $\beta$ -binding compound effective for prophylactic treatment or therapy is the same as that for therapeutic treatment or therapy.

### **EXAMPLES**

The following example describes specific aspects of the  
15 invention to illustrate the invention and provides a description of the present methods for those of skill in the art. The example should not be construed as limiting the invention, as the examples merely provide specific methodology useful in the understanding and practice of the invention and its various aspects.

#### **Example 1**

20 Transgenic mice that develop AD-related amyloidosis, (i.e., PS/APP mice; See, L. Holcomb et al., 1998, *Nature Med.*, 4(1):97-100) were used for the studies described in this Example to assess how peripheral sequestration of A $\beta$  affected brain A $\beta$  levels. The ganglioside GM1 was  
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A $\beta$  with great avidity, was administered peripherally to confirm the universality of the mechanism.

PS/APP mice were injected every two days for two weeks either with GM1 (number of mice =6) (15 mg/kg, ip), with gelsolin (number of mice =3) (60  $\mu$ g/kg, ip), or with vehicle, (phosphate buffered saline), (number =7), into the periphery at 9 weeks of age, an age when amyloid pathology in the brain is not visible. The mice were left for 1 week without injections (the "wash-out" period) and were then sacrificed at 12 weeks of age, an age when amyloid deposition has been initiated and measurable levels of A $\beta$  are present in the vehicle treated controls.

The levels of A $\beta$  in the peripheral blood were tested at three time points during the drug administration period (i.e., after 1 week of injections; after two weeks of injections; and after the wash-out period). Data for the third time point only are shown. The levels of A $\beta$  peptides (A $\beta$ 40 or A $\beta$ 42) in the brain and plasma were assessed by ELISA assay. All of the A $\beta$  in the brain (including A $\beta$  in plaques) was extracted in 70% formic acid (FA). The levels of A $\beta$  peptides in GM1 or gelsolin treated mice were compared with those of vehicle treated controls.

The results presented in Table 1 show that there was a statistically significant ( $p < 0.05$ ) decrease in both A $\beta$ 40 and A $\beta$ 42 in the FA-soluble brain fraction in GM1-treated mice compared with those in control animals. This correlates with a statistically significant increase in peripheral A $\beta$ 40 and A $\beta$ 42 at the same time point. For gelsolin, there was also a significant decrease in A $\beta$ 42, which correlated with a statistically significant increase in peripheral A $\beta$ , thus confirming that the general principle of A $\beta$  sequestration in the periphery being linked to reduction of A $\beta$  in the CNS

holds true for very different types of compounds that have the unifying property of being able to bind A $\beta$  in the blood.

**Table 1**

5 Changes of brain Abeta load [fmole/mg protein] in GM1- and Gelsolin-treated mice compared with controls (vehicle)

Treatment	n	Abeta40	Abeta42
Vehicle	7	3630 $\pm$ 30	4100 $\pm$ 200
GM1	6	2147 $\pm$ 226	2990 $\pm$ 410
	%	59	73
P-value	p	0.005**	0.031*
Gelsolin	3	3473 $\pm$ 673	2473 $\pm$ 167
	%	96	60
P-value	p	0.502	0.021*

10 \*: results statistically significant to the 5% level (p=0.05);

\*\*: results statistically significant to the 1% level (p=0.001).

10

The results presented in Table 2 show the changes in plasma load of both A $\beta$ 40 and A $\beta$ 42 in GM1-treated mice compared with control (vehicle) animals. As can be observed, there was a significant increase in plasma Abeta levels. For GM1, both Abeta40 and Abeta42 are increased; for gelsolin, Abeta42 is increased to a greater extent than is Abeta40. Thus, the effect with gelsolin may reflect a different preference for Abeta42 over Abeta40.

15

**Table 2**

20 Changes of plasma Abeta load [fmole/ml plasma] in GM1 treated mice compared with controls (vehicle)

Treatment	n	Abeta40	Abeta42
Vehicle	7	100 $\pm$ 21	100 $\pm$ 7
GM1	6	177 $\pm$ 29	124 $\pm$ 11
	p	0.021**	0.036*
Gelsolin	3	218 $\pm$ 87	153 $\pm$ 35
	p	0.016**	0.039*

20 \*: results statistically significant to the 5% level (p=0.05);

\*\*: results statistically significant to the 1% level (p=0.001).

Neither GM1 nor gelsolin is known to cross into the brain from the periphery to any degree. In addition, as part of the studies related to those described in this example, GM1 was introduced directly into the brain of transgenic mice, but no change in A $\beta$  levels was observed. Thus, the results indicate that the effects of GM1 administration in the test mice is due to the sequestration of A $\beta$  in the periphery, thereby leading to a change in dynamics between brain and peripheral A $\beta$  transport. This is the first time that such a result has been shown for a peripherally administered compound that is not an antibody. As such, the invention affords a significant advantage to the art by describing and promoting A $\beta$ -binding compounds that require neither penetration of the brain nor the evocation of an immune response, which are potentially harmful and ineffective ways to modulate the risk of AD in human patients.

It will be appreciated that the use of GM1 or gelsolin in the present example is not limiting to the types of compounds considered to be suitable for use in the present invention. Indeed, in accordance with this invention, any A $\beta$  binding molecule can have the same effect following peripheral administration, thus providing a powerful treatment and therapeutic for AD sufferers, as well as those afflicted with other amyloidoses, e.g., amyloid angiopathy.

### Example 2

In this Example, as in Example 1, transgenic mice that develop AD-related amyloidosis, (i.e., PS/APP mice; See, L. Holcomb et al., 1998, *Nature Med.*, 4(1):97-100) were used to assess the A $\beta$ -binding compound chrysamine G (CG) in the peripheral sequestration of A $\beta$  according to this invention, and to determine how the peripheral sequestration of A $\beta$  by this compound affected brain A $\beta$  levels. CG is known to bind A $\beta$  strongly, and is less brain permeable than GM1.

PS/APP mice at 10 weeks of age were injected once either with CG (number of mice=3, dosage: 20 mg/kg) or vehicle (phosphate buffered saline, number of mice=2) into the blood stream. Blood samples were collected prior to treatment (injection) and post-treatment at 10 minutes, 2.5 hours, 5 hours and 25 hours after injection. Blood A $\beta$  levels were compared between pre-treatment versus post-treatment at 10 minutes, 2.5, 5 and 25 hours after injection. The levels of A $\beta$  peptides (A $\beta$ 40 or A $\beta$ 42) in the plasma were assessed by ELISA immunoassay. The levels of A $\beta$  peptides in the periphery, i.e., plasma, of CG treated mice were compared with plasma A $\beta$  levels in pre-treatment mice at various time points.

Changes in plasma A $\beta$  levels after injection with CG were compared with pre-treatment plasma A $\beta$  levels as shown in Table 3.

**Table 3**

15

Changes in plasma A $\beta$ levels after injection with CG	
Hours after injection	A $\beta$ 42 level [% of pre-treatment time point]
2.5	110 $\pm$ 8 ( $p=0.232$ )
5	125 $\pm$ 8 ( $p=0.049$ )
7.5	125 $\pm$ 7 ( $p=0.034$ )
24	112 $\pm$ 13 ( $p=0.341$ )
48	111 $\pm$ 10 ( $p=0.256$ )

The results presented in Table 3 show that there was a statistically significant ( $p<0.05$ ) increase in A $\beta$ , as represented by A $\beta$ 42 determination, in the plasma of the mice injected with CG at 5 and 7.5 hours after injection. Changes in plasma load of A $\beta$ 42 after injection with CG were compared to the plasma A $\beta$  level at pre-treatment time points. As can be observed, there was a significant increase in plasma A $\beta$  levels after injection of CG.

Following a one-week wash out period, the effect in brain A $\beta$  level after continuous injection was examined. PS/APP mice were injected every day for one week into the periphery, with either CG (number of mice = 3) (20 mg/kg, ip,) or vehicle (phosphate buffered saline) (number of mice = 2), at 11 weeks of age, an age when amyloid pathology in the brain is not visible. The mice were sacrificed at 12 weeks of age, an age when amyloid deposition has been initiated and measurable levels of A $\beta$  are present in the vehicle treated controls.

The levels of A $\beta$  in the peripheral blood were tested at the end of the administration period. The levels of A $\beta$  peptides (e.g., A $\beta$ 40 or A $\beta$ 42) in the brain and plasma were assessed by ELISA assay. All of the A $\beta$  in the brain (including A $\beta$  in plaques) was extracted in 70% formic acid (FA). The levels of A $\beta$  peptides in CG treated mice were compared with those of vehicle treated controls. The results presented in Tables 4 and 5 show that there was a statistically significant ( $p < 0.05$ ) decrease in A $\beta$ 40 and/or A $\beta$ 42 in the FA-soluble brain fraction in CG-treated mice compared with those in control animals tested 1 week after injection. This correlates with a statistically significant increase in peripheral A $\beta$ 40 and A $\beta$ 42 at the same time point.

**Table 4**

Change of plasma A $\beta$  levels 1 week following injection with CG

	Plasma A $\beta$ 42 level [% of control]
Vehicle	100 $\pm$ 9
CG treated	331 $\pm$ 10
	( $p=0.0081$ )

**Table 5**Change of brain A $\beta$  1week following injection with CG

	A $\beta$ 40 [fmol/ml]	A $\beta$ 42 [fmol/ml]
Vehicle	926 $\pm$ 26	1209 $\pm$ 292
CG	541 $\pm$ 50	540 $\pm$ 271
	(P=0.0031)	(P=0.2038)*

\*: Because of data variation, A $\beta$ 42 was not statistically significant, p=0.20.

5                   The method of the present invention for determining elevated levels of A $\beta$  in the periphery for the purposes of diagnosing, screening, or monitoring patient treatment, treatment outcome, or the course and/or severity of amyloid-related disease in an individual preferably involves a pretreatment or baseline value for assessing peripheral elevation of A $\beta$

10                   levels in the individual undergoing testing. In the examples presented herein, an elevation of plasma Abeta was compared by percentage pre-treatment time point of an individual animal. Similar comparative assessments of pretreatment and treatment Abeta levels can be employed for the testing of other mammals, including humans, particularly because the

15                   range of Abeta levels can be large between and among individuals. As a nonlimiting guide, a representative non-elevated level of Abeta in human plasma (e.g., periphery) is about 25%, as determined experimentally (e.g., Mehta et al., 2000, *Ibid.*).

20                   The contents of all patents, patent applications, published PCT applications and articles, books, references, reference manuals and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the invention pertains.

25                   As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present invention, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive

and illustrative of the present invention. Many modifications and variations of the present invention are possible in light of the above teachings.

**WHAT IS CLAIMED IS:**

1. A method of treating amyloid beta (A $\beta$ )-associated disease, comprising administering an amyloid beta (A $\beta$ )-binding agent in the periphery of an individual in need thereof, wherein said agent binds to A $\beta$  in the periphery, sequesters A $\beta$  in the periphery and concomitantly decreases A $\beta$  levels in the brain of the individual undergoing treatment, in the absence of immunomodulating agents.
2. The method according to claim 1, wherein the amyloid beta (A $\beta$ )-associated disease is selected from Alzheimer's disease,  $\beta$ -amyloid related problems of Down's syndrome, vascular dementia (cerebral amyloid angiopathy) and amyloidosis.
3. The method according to claim 1, wherein the amyloid beta (A $\beta$ )-binding agent is selected from the group consisting of GM1 ganglioside, gelsolin, an A $\beta$  imaging agent, a  $\beta$ -sheet breaker, a  $\beta$ -sheet formation inhibitor and a derivative of an amyloid beta (A $\beta$ )-staining dye.
4. The method according to claim 1, wherein the derivative of the amyloid beta (A $\beta$ )-staining dye is 1,4-bis(3-carboxy-4-hydroxyphenylethenyl)-benzene and 5,5'-{(1,1' biphenyl)-4, 4'-diylbis(azo)} bis {2-hydroxybenzoic acid} disodium salt (chrysamine-G).
5. The method according to claim 1, wherein amyloid beta (A $\beta$ )-binding agent is virtually brain impermeable.
6. A method for sequestering A $\beta$  in the periphery comprising blood or blood components of an individual in need thereof, comprising:
  - a) administering an agent having binding affinity for amyloid beta (A $\beta$ ) in the periphery of the individual in need thereof;

b) sequestering A $\beta$  in the periphery, thereby concomitantly decreasing A $\beta$  levels in the brain of the individual.

7. The method according to claim 6, wherein the agent having binding affinity for amyloid beta (A $\beta$ ) is administered in the absence  
5 of immunomodulating agents or brain penetrance.

8. The method according to claim 6, wherein the individual is suffering from an amyloid beta (A $\beta$ )-associated disease.

9. The method according to claim 8, wherein the amyloid beta (A $\beta$ )-associated disease is selected from Alzheimer's disease,  $\beta$ -  
10 amyloid related problems of Down's syndrome, vascular dementia (cerebral amyloid angiopathy) and amyloidosis.

10. The method according to claim 6, wherein the agent having binding affinity for amyloid beta (A $\beta$ ) is selected from the group consisting of GM1 ganglioside, gelsolin, an A $\beta$  imaging agent, a  $\beta$ -sheet  
15 breaker, a  $\beta$ -sheet formation inhibitor and a derivative of an amyloid beta (A $\beta$ )-staining dye.

11. The method according to claim 10, wherein the derivative of the amyloid beta (A $\beta$ )-staining dye is 1,4-bis(3-carboxy-4-hydroxyphenylethenyl)-benzene and 5,5'-((1,1' biphenyl)-4, 4'-diylbis(azo))  
20 bis {2-hydroxybenzoic acid} disodium salt (chrysamine-G).

12. A method of monitoring the effectiveness of drug treatment of beta-amyloid related diseases, comprising:

- (a) assessing levels of A $\beta$  in the periphery of a recipient of the drug treatment; and  
25 (b) determining an elevation of the levels of A $\beta$  in the periphery of the recipient.

13. The method according to claim 12, wherein the levels (A $\beta$ ) are assessed by an assay that detects A $\beta$ .

14. The method according to claim 13, wherein the assay is (i) a radioisotopic immunoassay or (ii) a non-isotopic immunoassay.

5 15. The method according to claim 14, wherein the non-isotopic immunoassay is selected from a fluorescent immunoassay, a chemiluminescent immunoassay, or an enzymatic immunoassay (ELISA).

16. The method according to claim 12, wherein the drug treatment comprises an agent having binding affinity for amyloid beta (A $\beta$ ).

10 17. The method according to claim 16, wherein the agent is selected from the group consisting of GM1 ganglioside, gelsolin, an A $\beta$  imaging agent, a  $\beta$ -sheet breaker, a  $\beta$ -sheet formation inhibitor and a derivative of an amyloid beta (A $\beta$ )-staining dye.

15 18. The method according to claim 17, wherein the derivative of the amyloid beta (A $\beta$ )-staining dye is 1,4-bis(3-carboxy-4-hydroxyphenylethenyl)-benzene and 5,5'-((1,1' biphenyl)-4, 4'-diylbis(azo)) bis {2-hydroxybenzoic acid} disodium salt (chrysamine-G).

19. The method according to claim 12, wherein the monitoring occurs at about 1-25 days following administration of drug to the  
20 recipient.

20. The method according to claim 12, wherein the monitoring occurs at about 5-10 days following administration of drug to the recipient.

21. A method of treating amyloidosis in a subject, said  
25 method comprising administering in the periphery of said subject an amyloid

beta (A $\beta$ ) binding agent, said agent selected from the group consisting of GM1 ganglioside, gelsolin, an A $\beta$  imaging agent, a  $\beta$ -sheet breaker, a  $\beta$ -sheet formation inhibitor and a derivative of an amyloid beta (A $\beta$ )-staining dye, for a time and under conditions suitable for the agent to bind amyloid  
5 beta (A $\beta$ ), sequester amyloid beta (A $\beta$ ) in the periphery and decrease amyloid beta (A $\beta$ ) levels in the brain of the individual.

22. The method according to claim 21, wherein the derivative of the amyloid beta (A $\beta$ )-staining dye is 1,4-bis(3-carboxy-4-hydroxyphenylethenyl)-benzene and 5,5'-{(1,1' biphenyl)-4, 4'-diylbis(azo)}  
10 bis {2-hydroxybenzoic acid} disodium salt (chrysamine-G).